Genetic Dissection of Seed Production Traits and Identification of a Major-Effect Seed Retention QTL in Hybrid *Leymus* (Triticeae) Wildryes

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ABSTRACT

Many native grasses display seed shattering and other seed production problems. Basin wildrye [*Leymus cinereus* (Scribn. & Merr.) Á. Löve] and creeping wildrye [*L. triticoides* (Buckley) Pilg.] are perennial Triticeae grasses native to western North America. In this study, variation in the number of florets per inflorescence, percent seed set, seed mass, seed shattering, and seed dormancy was measured in clonally replicated multiyear field evaluations of 164 progeny derived from a backcross between a creeping × basin wildrye hybrid and a true creeping wildrye tester. Genomewide quantitative trait locus (QTL) scans detected independent gene effects for all five traits and one pleiotropic QTL, located on Triticeae homeologous chromosome group 6, associated with major seed shattering (43.1% R^2, 26.9 log of odds likelihood ratio [LOD]) and seed dormancy (24.1% R^2, 10.6 LOD) effects. We hypothesize that a dominant basin wildrye gene creates an abscission that promotes seed disarticulation but hinders seed development and seed ripening in the creeping wildrye backcross family. Alignments of *Leymus* chromosome 6 expressed sequence tag markers to the rice (*Oryza*) genome reveal well-defined correspondence of *Leymus* wildrye and American wildrice (*Zizania palustris* L.) seed shatter QTLs.

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Abbreviations: CM, Chapalote maize; EST, expressed sequence tag; LOD, log of odds likelihood ratio statistic; MQM, multiple-QTL model; QTL, quantitative trait locus; RFLP, restriction fragment length polymorphism; rMQM, restricted MQM; RP, Reventador maize.

Native perennial grasses have many uses in agriculture, conservation, and landscaping. However, high-quality seed for native grasses and other wild plants is difficult and expensive to produce (Dunne and Dunne, 2003; Cole and Johnston, 2006). Many native plants display active mechanisms of seed shattering that create a multitude of problems when these species are cultivated for seed (Berdahl and Frank, 1998; Kennard et al., 2002; Lemke et al., 2003; Bedane et al., 2005; Wang et al., 2008). Seed shattering can dramatically reduce yield if harvests are delayed for any reason, but the seed quality can be compromised if harvested before physiological maturity. Moreover, many natural plant populations display variation in phenology that can complicate harvest of native plant seeds.

Basin wildrye [*Leymus cinereus* (Scribn. & Merr.) A. Löve] and creeping wildrye [*L. triticoides* (Buckley) Pilg.] are closely related but morphologically divergent perennial Triticeae grasses native to western North America. Both species are allogamous (Jensen et al., 1990), hybridize in nature (Barkworth et al., 2007), and show considerable genetic variation within and among natural populations. Two full-sib backcross populations derived from

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two basin wildrye and creeping wildrye hybrids and one creeping wildrye recurrent parent genotype have been used to construct high-density linkage maps (Wu et al., 2003); identify quantitative trait loci (QTLs) controlling plant height, rhizome proliferation, flowering, and forage quality traits (Larson et al., 2006, 2007); and to investigate other functionally important traits including seed shattering and germination as described in this report.

Basin wildrye is an unusually tall caespitose grass, capable of growing up to 3 m high, and is considered one of the largest native grasses in western North America (Lesperance et al., 1978; Evans and Young, 1983; Cash et al., 1998). Basin wildrye displays deep and extensive fibrous root systems (Reynolds and Fraley, 1989; Abbott et al., 1991), adaptation to saline and nonsaline soils (Lesperance et al., 1978; Young and Evans, 1981; Miller et al., 1982; Roundy, 1983; Roundy et al., 1985), and unusual resource efficiency with extended seasonal photosynthetic capacity (Anderson et al., 1995). Basin wildrye is widely distributed throughout western North America (Barkworth et al., 2007) and natural germplasm sources of basin wildrye are cultivated for seed used in rangeland revegetation and mine reclamation (Cash et al., 1998; Richards et al., 1998; Neuman and Schafer, 2006). Basin wildrye is also considered valuable native forage grass in the Great Basin and other regions of western North America (Ganskopp et al., 1996, 1997; Cash et al., 1998; Ganskopp and Bohnert, 2001), which may be particularly useful as winter feedstock (Majerus, 1992; Jensen et al., 2002). Basin wildrye seeds are effectively nondormant at dispersal (Young and Evans, 1981; Evans and Young, 1983; Meyer et al., 1995). However, the production and quality of basin wildrye seed can be problematic because this species displays a strong propensity to shatter as the seed matures.

Creeping wildrye is a strongly rhizomatous grass (Larson et al., 2006) that is well adapted to saline meadows and cultivated as a saline biomass crop (Suyama et al., 2007a,b). Creeping wildrye is a relatively poor seed producer and is notorious for having extensive seed dormancy characteristics, which limit its use in rangeland revegetation and agriculture (Knapp and Wiesner, 1978). However, creeping wildrye is effectively nonshattering and shows very good seed retention.

Both basin wildrye and creeping wildrye have similar distichous spikes with one or more spikelets per node and three to seven florets per spikelet, except that basin wildrye has longer spikes with more nodes and more spikelets per node than creeping wildrye (Barkworth et al., 2007). Seed disarticulation in *Leymus* and other perennial Triticeae genera, including *Agropyron*, *Pseudoroegneria*, *Pascopyrum*, most *Thinopyrum* species, and most *Elymus* species, generally occurs above the glumes and beneath the florets (Barkworth et al., 2007), which is consistent with our observations of basin wildrye and the creeping × basin wildrye hybrids. After the floret disarticulates from the spikelet rachila, the resulting diaspore is defined by a caryopsis with lemma and palea attached. In contrast, some perennial Triticeae genera including *Psathyrostachys*, some *Thinopyrum* species, and some *Elymus* species disarticulate in the rachises, which is also common among annual grasses in the genera *Aegilops*, *Hordeum*, *Secale*, and *Triticum* (Barkworth et al., 2007). However, all of these Triticeae genera and species show considerable variation in their propensity to shatter.

Seed retention was a fundamentally important step in the domestication of cereal crops. The number and location of genetic factors controlling seed shattering and other key differences between cultivated and wild forms of maize (*Zea mays* ssp. *mays* L. × *Z. mays* ssp. *parviglumis* Iltis & Doebley) (Doebley and Stec, 1993), sorghum [*Sorghum haplense* L. and *S. bicolor* (L.) Moench] (Paterson et al., 1995b), rice (*Oryza sativa* L., *O. nivara* Sharma & Shastry, and *O. rufipogon* Griff.) (Cai and Morishima, 2000; Li et al., 2006; Konishi et al., 2006), pearl millet [*Pennisetum glaucum* ssp. *glaucum* (L.) R. Br. × *P. glaucum* ssp. *monodii* (Maire) Brunken] (Poncet et al., 2002), American wildrice (*Zizania palustris* L.) (Kennard et al., 2002), barley (*Hordeum vulgare* L.) (Komatsuda et al., 2004), and wheat (*Triticum* spp.) (Watanabe et al., 2002; Li and Gill, 2006) have been analyzed and compared, as quantitative trait loci (QTL), using molecular genetic markers. Evidence of orthology involving one or more major seed shattering gene effects was inferred between maize chromosome 1 and 5, rice chromosome 9, and sorghum C (Paterson et al., 1995a); maize chromosome 4, rice chromosome 2, and American wildrice chromosome 2 (Paterson et al., 1995a; Kennard et al., 2002); maize chromosome 3 and 8, rice chromosome 1, and pearl millet chromosome 6 (Cai and Morishima, 2000; Poncet et al., 2002); and Triticeae homeologous group 3 chromosomes (Watanabe et al., 2002). However, at least two different brittle rachis genes have been mapped on Triticeae homeologous group 3 (Li and Gill, 2006).

The main objectives of this investigation were to determine the number and location of QTLs controlling differences between creeping wildrye seed retention and basin wildrye seed shattering traits, and compare the genomic location of seed shattering QTLs between *Leymus* and other grass species. A second objective was to determine if seed shattering in *Leymus* is associated with other important seed production or seed quality traits (e.g., seed mass, seed dormancy, seeds per inflorescence, and fertility), which are also known to differ among these *Leymus* wildrye species.

MATERIALS AND METHODS

Plant Materials and Genetic Maps

Wu et al. (2003) constructed the first molecular genetic map of the *Leymus* TTC1 QTL mapping family. The 164-sib TTC1

family was derived from a cross of one *L. triticoides* Acc:641 plant (T-tester) pollinated by one *L. triticoides* Acc:641 × *L. cinereus* Acc:636 hybrid plant (TC1). The *L. triticoides* Acc:641 × *L. cinereus* Acc:636 TC1 hybrid was backcrossed the same *L. triticoides* Acc:641 accession, but not the exact same *L. triticoides* plant. The TTC1 map includes 1069 amplified fragment length polymorphism markers and 53 anchor loci in 14 linkage groups spanning 2001 cM (Fig. 1), which covers virtually all of the 14 chromosomes in this allotetraploid ($2n = 4x = 28$) *Leymus* population (Wu et al., 2003; Larson et al., 2006). The 14 linkage groups (LG1a, LG1b, LG2a, LG2b, LG3a, LG3b, LG4Ns, LG4Xm, LG5Ns, LG5Xm, LG6a, LG6b, LG7a, and LG7b) were numbered according to the seven homeologous chromosome groups of the Triticeae cereals as described by Wu et al. (2003). The *Leymus* expressed sequence tag (EST) markers, integrated consensus maps, and *Leymus*-6a/*Oryza* chromosome 2 (Fig. 2) and *Leymus* LG3b/*Oryza* chromosome 1 (Fig. 3) alignments were described by Bushman et al. (2008) and Kaur et al. (2008), respectively. Additional reference markers associated with the American wildrice seed shatter QTL (Kennard et al., 2000, 2002), rice chromosome 2 consensus QTL (Paterson et al., 1995a), the *teosinte glume architecture1* (*tga1*) gene (Wang et al., 2005), and the rice *qSH1* gene (Konishi et al., 2006) were aligned to the *Oryza* references maps (see "Results and Discussion" below) using the *Oryza* genome (Dickson and Cyranoski 2001; Goff et al., 2002; Yu et al., 2002) BLAST (Altschul et al., 1997) available at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi (verified 30 Oct. 2008).

An advanced backcross family (TTTC1.168) was used for QTL validation. The TTTC1.168 family was derived from a cross of one recombinant TTC1 genotype (TTC1.168) backcrossed to the *L. triticoides* Acc:641 T-tester genotype. The TTC1.168 line was heterozygous for *L. cinereus* and *L. triticoides* marker alleles between 0 and 92 cM and homozygous for *L. triticoides* marker alleles between 92 and 164 cM relative to the LG6a integrated consensus map (Fig. 2). This family was genotyped using six LG6a markers as shown in Fig. 2.

Phenotypic Evaluations

The TTC1 field evaluations were established in 2001 with two clonally replicated randomized complete blocks on 2-m centers at the Utah Agriculture Experiment Station, Richmond Farm, as described by Larson et al. (2006). The second-generation TTTC1.168 family was planted in 2003 with two clonally replicated randomized complete blocks on 3-m centers in North Logan, UT.

Seed dormancy and seed mass measurements were based on subsamples of 100 seeds harvested from each TTC1 plot in 2004 and 2005. All seed dormancy and seed mass measurements were obtained using a similar protocol where field-harvested seed materials were stored in loose paper bags in a temperature-controlled greenhouse, optimized for maintenance of live plants. Once all plots were harvested, seed was threshed and stored in coin envelopes at room temperature. Resulting seed samples, from each plot, were dusted with tetramethylthiuram disulfide and placed on blue germination blotter paper (Anchor Paper, St. Paul, MN) moistened with distilled water in 10.95 by 10.95 by 3.49-cm acrylic germination boxes with friction lids (Cont 156C; Hoffman Manufacturing, Inc., Jefferson, OR). Sets of eight germination boxes were wrapped in a light plastic bag to help maintain moisture within each box. Seeds were imbibed in these germination boxes for 3 d in the laboratory, stratified at 4°C for another 14 d in a dark cold-room chamber, and then allowed to germinate for up to 28 d in dark plant-growth chambers maintained on a diurnal cycle of 16 h at 15° and 8 h at 15°. Seeds were considered germinated when either seedling root or shoots were visible.

Seed dormancy and seed mass traits in the TTC1 family were evaluated in 2004, the first year of good seed production, using inflorescence material that was periodically clipped as each plant senesced over the course of about 3 wk (4–6 August, 9–10 August, 20 August, and 24–25 August). Seed germination tests were performed once for each field plot in two sets corresponding to each of the two field replications, with the first and second sets (reps) beginning on 17 Dec. 2004 and 7 Jan. 2004, respectively. Germinated seeds were periodically counted and removed after 7, 14, 21, and 28 d.

Percent seed set, percent shattering florets, seed mass, and seed dormancy traits were evaluated in 2005 by periodically collecting seeds and florets as they naturally shattered over the course of 54 d (8 August, 15 August, 23 August, 30 August, 6 September, 19 September, and 30 September). More specifically, shattering material was repeatedly collected from each plot by bending culms over, approximately 180°, and nondestructively knocking clumps of inflorescence around the insides of a 19-L pail. All remaining nonshattering inflorescence material was collected on 30 September, after all plants had completely senesced and ceased shattering. Estimates of the number of shattering and nonshattering seeds were determined based on measurements of the mass of 100 seeds from each plot. Likewise, estimates of number of empty florets, in shattering and nonshattering material, were based on measurements of the average mass of 100 empty florets from 10 plots. Thus, estimates of the number of shattering and nonshattering florets were based on the total number of seeds and empty florets from each fraction. Percent shattering florets was determined based on the ratio of shattering florets to nonshattering florets. Percent seed set was estimated based on the overall ratio of seeds to empty florets collected from each plot. Germination tests were performed twice for each field plot using two incomplete sets of the first field replication block begun on 20 Jan. 2006 and 27 Jan. 2006, two incomplete sets of the second field replication block begun on 24 Feb. 2006 and 3 March 2006, another two incomplete sets of the first field replication block begun on 31 Mar. 2006 and 7 Apr. 2006, and another two incomplete sets of the second field replication block begun on 17 Dec. 2004 and 7 Jan. 2004. Germinated seeds were periodically counted and removed after 1, 2, 4, 8, 16, and 24 d.

Percent shattering and the number of florets per inflorescence were measured using subsamples of five inflorescences clipped from each TTC1 plot on 10 Aug. 2006. The number of florets per inflorescence was determined by multiplying the average (no. florets spikelet^{-1})(no. spikelets node^{-1})(nodes 6 cm^{-1})(cm length inflorescence^{-1}). Shattering in the 2006 TTC1 evaluation was subsequently determined by the percentage of florets that shattered during manual agitation of each inflorescence in the laboratory.

Seed shattering was evaluated in the advance backcross TTTC1.168 family in 2005 by agitating subsamples of 10 intact

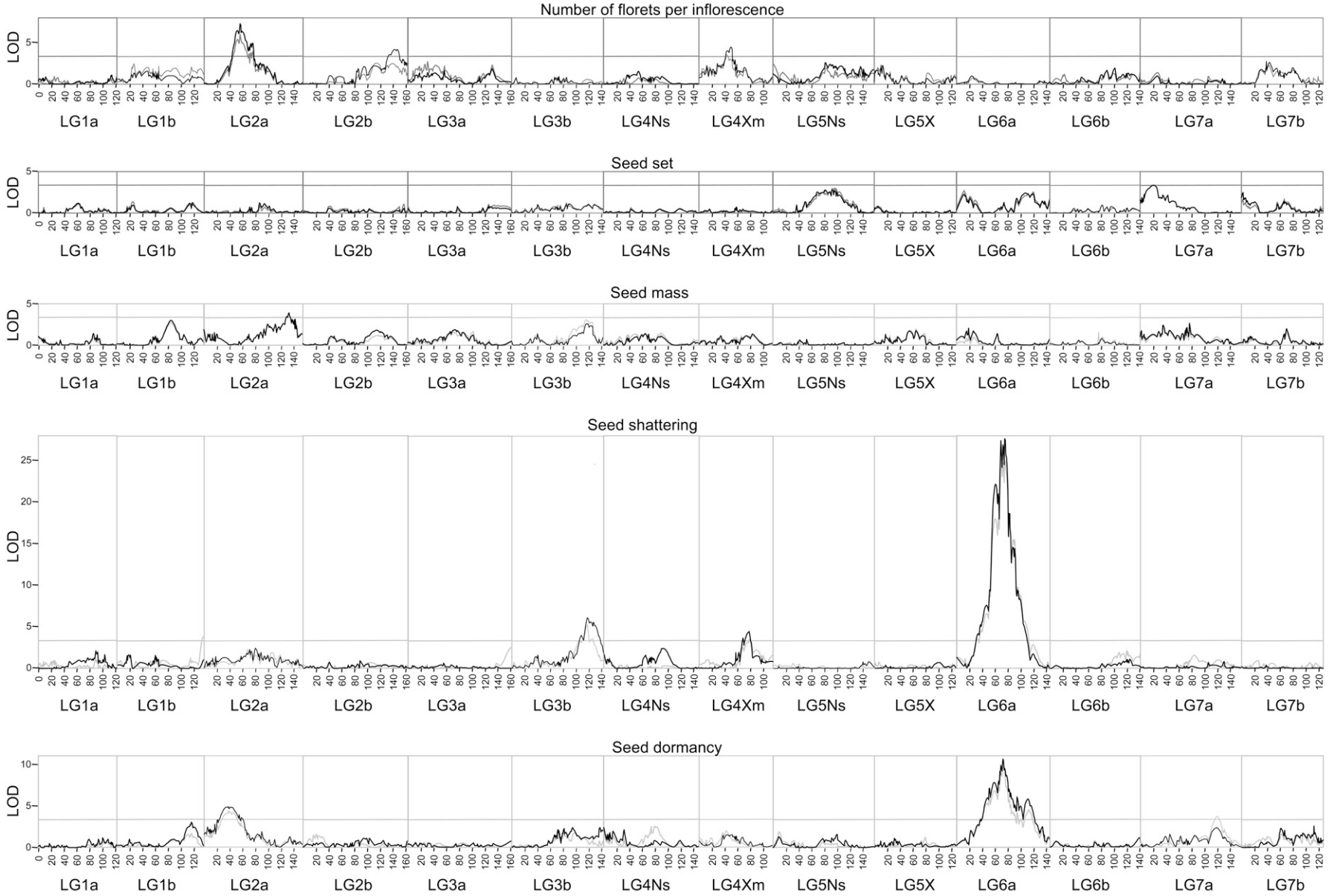

Figure 1. Genomewide quantitative trait loci (QTLs) scans with log of odds likelihood ratio (LOD) value on vertical axis and linkage group (LG) position (in cM) for the number of florets per inflorescence, percent seed set, seed mass, percent seed shatter, and seed dormancy using the *Leymus* TTC1 family and high-density molecular genetic linkage maps described by Wu et al. (2003). The approximate 5% genomewide QTL significance threshold, 3.3 LOD, is shown as a gray horizontal line for each trait scan. The main effect and restricted multiple QTL model scans are shown in gray and black, respectively.

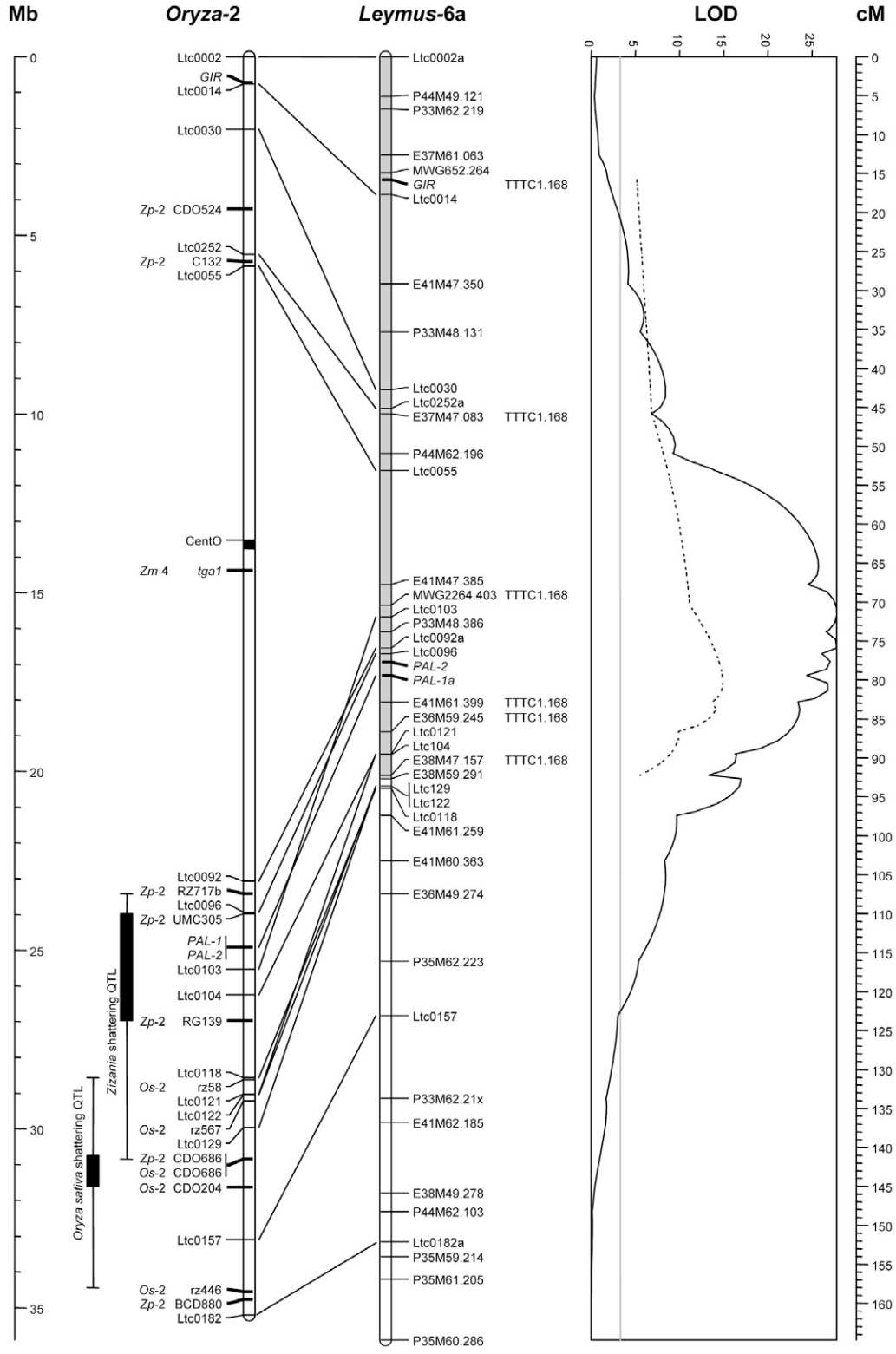

Figure 2. Seed shattering quantitative trait loci (QTLs) scans in the *Leymus* TTC1 (solid line) and *Leymus* TTTC1.168 advance backcross (dashed line) populations using the *Leymus* linkage group 6a expressed sequence tag integrated consensus map and rice chromosome 2 sequence alignments described by Bushman et al. (2008). The approximate 5% genomewide QTL significance threshold, 3.3 log of odds likelihood ratio (LOD), is shown as a gray horizontal line. The recombination distances for *Leymus* linkage group 6a are shown (in cM) on the right side of figure, whereas the physical distances on *Oryza* chromosome 2 are shown (in Mb) on the left side of figure. Markers used for QTL scans of the TTTC1.168 advance backcross population are indicted by text on the right side of the *Leymus* LG6a genetic map. Additional markers used for *Zizania palustris* (Kennard et al. 2002) and *Oryza sativa* (Paterson et al. 1995a) QTL marker alignments are indicated text (*Zp-2* and *Os-2*, respectively) on the left side of the *Oryza* chromosome 2 physical map. A *Zea mays* reference gene, *teosinte glume architecture 1* (*tga1*) (Dorweiler et al., 1993; Wang et al. 2005), located near maize seed disarticulation QTL (Doebley and Stec, 1993; Paterson et al., 1995a) is also annotated by text (*Zm-4*) on the *Oryza* chromosome 2 physical map.

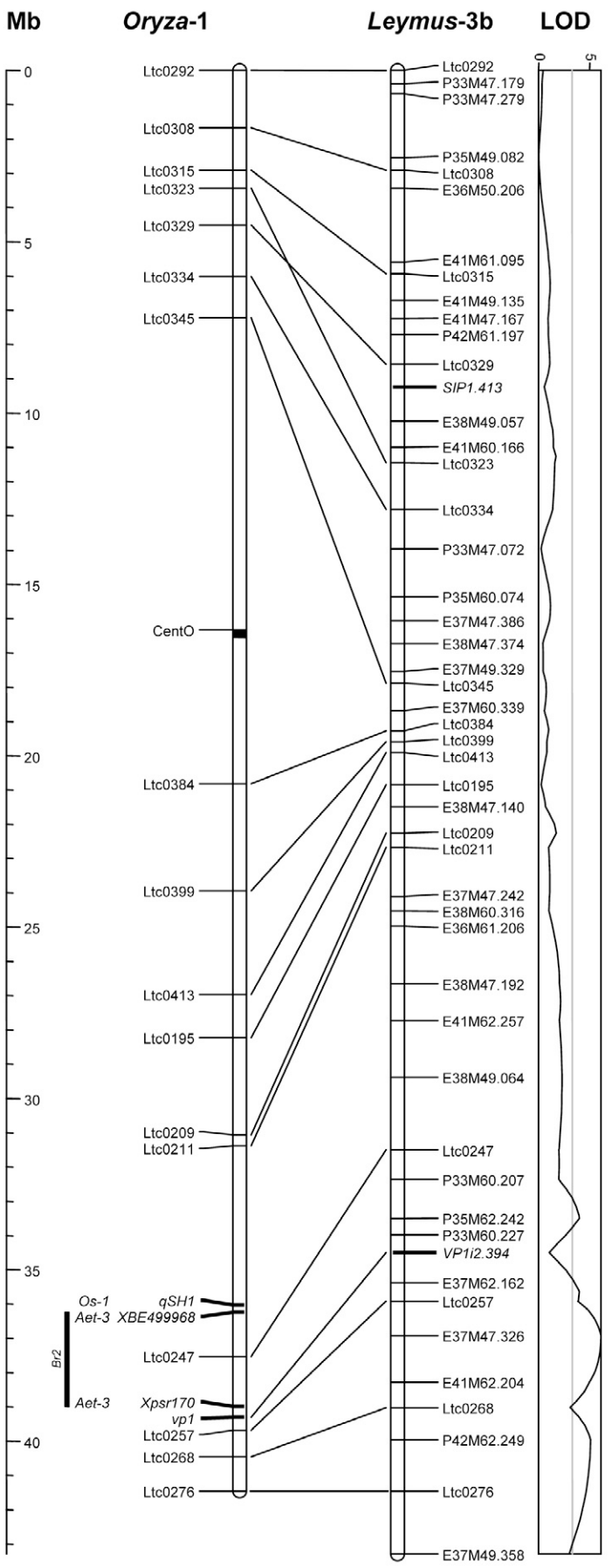

Figure 3. Seed shattering quantitative trait loci (QTLs) scans in the *Leymus* TTC1 population using the *Leymus* linkage group 3b expressed sequence tag integrated consensus map and *Oryza* chromosome 1 sequence alignments described by Kaur et al. (2008). The approximate 5% genomewide QTL significance threshold, 3.3 log of odds likelihood ratio (LOD), is shown as a gray horizontal line. The recombination distances (in cM) for *Leymus* linkage group 3b are shown on the right side of figure, whereas the physical distances (in megabases [Mb]) on *Oryza* chromosome 1 are shown on the left side of figure. Additional markers flanking the *Aegilops tauschii* spikelet disarticulation gene (*Br2*) are indicated by *Aet-3* text on the left side of the *Oryza* chromosome 1 physical map. The *Oryza sativa* gene controlling the rice qSH1 seed shattering QTL (Konishi et al. 2006) is annotated by text (*Os-1*) on the *Oryza* chromosome 1 physical map.

inflorescences, on the plant, and rating each plot as 0 (no shattering), 1 (~25% shattering), 2 (~50% shattering), 3 (~75% shattering), and 4 (complete or nearly complete shattering). This TTTC1.168 shattering evaluation was performed on a one-pass basis between 9 Sept. 2005 and 14 Sept. 2005 (block 1) and between 15 Sept. 2005 and 19 Sept. 2005 (block 2).

Statistical Analyses

Seed dormancy was measured by the number of days required to reach 50% germination (DRG_{50}) as determined by logistic regression using the proportion of germinated seeds as the dependent variable and the number of days required for germination as the independent variable using the Statistical Analysis Software PROBIT procedure (SAS Institute Inc., Cary, NC) for each germination test. All trait means and standard errors were calculated using least squares means from the SAS GLM procedure based on measurements of different subsamples, field replications, and evaluation years.

A sequential and reiterative procedure of QTL detection was performed using the MapQTL version 5.0 package (Van Ooijen, 2004). Genomewide interval mapping (Lander and Botstein, 1989) was performed in 1-cM increments to identify putative QTLs and possible cofactors used in a multiple-QTL model (MQM) (Jansen, 1993, 1994; Jansen and Stam, 1994). Markers with the highest log-likelihood ratios (i.e., log of odds likelihood ratio [LOD] test statistics) for each QTL (no more than one per chromosome) were selected as the initial set of possible MQM cofactors. A backward elimination procedure was applied to this initial set of cofactors using a conservative significance level of 0.001 to ensure the independence of each cofactor. A reiterative process of restricted MQM (rMQM) mapping, which excludes any syntenous cofactors (i.e., cofactors located on the same linkage group that is being scanned), was used to refine the location of rMQM cofactors (QTLs) and identify new rMQM cofactors

(QTLs). Permutation analyses (Churchill and Doerge, 1994), implemented in MapQTL (Van Ooijen, 2004), with 1000 replications were used to determine the genomewide 5% significance level for QTL LOD interval mapping. Map diagrams and QTL graphs were constructed using MapChart 2.2 (Voorrips, 2002).

RESULTS AND DISCUSSION

Trait Means and Correlations

Compared with *L. triticoides* Acc:641 reference samples and *L. triticoides* T-tester, the *L. cinereus* Acc:636 reference samples showed greater seed mass, more seed shattering, more seeds per inflorescence, and less seed dormancy (Table 1). The TC1 hybrid showed even greater seed mass and more seed shattering compared with either of the *L. cinereus* Acc:636 or *L. triticoides* Acc:641 parental accessions or the *L. triticoides* T-tester genotype. The TC1 hybrid produced more seeds per inflorescence than the T-tester and other *L. triticoides* Acc:641 reference plants, but fewer seeds per inflorescence than *L. cinereus* Acc:636. Seed dormancy in the TC1 hybrid was also intermediate between *L. cinereus* Acc:636 and *L. triticoides* Acc:641 (including T-tester) in 2004, but slower than *L. cinereus* Acc:636 or *L. triticoides* Acc:641 (including T-tester) in 2005. The *L. cinereus* Acc:636 and *L. triticoides* Acc:641 reference samples showed similar levels of fertility, but percent seed set was diminished in the TC1 hybrids and the TTC1 progeny. Nevertheless, the TTC1 family showed at least twofold variation in the range of all trait values (Table 1) with high- and low-transgressive variation relative to the TC1 and T-tester parents or the *L. cinereus* Acc:636 and *L. triticoides* Acc:641 reference samples.

Significant positive correlations between replications were observed for all traits (Table 2). Correlations between measurements taken over different years for seed mass, seed dormancy, and seed shattering were similar to the average correlation coefficients between replications within years (Table 2). These results indicate that trait means combined over replications and years provide a robust measure of genetic variation in the TTC1 family. The strongest relationship between different traits involved a significant positive correlation between percent seed shattering and seed dormancy in 2005 (Table 2). All other possible relationships between different traits were weak or not significant.

Genomewide QTL Analyses

Permutation analyses for all five traits measured in the TTC1 family indicated a threshold of 3.3 LOD as a close approximation for a genomewide 5% significance level. Genomewide QTL scans detected significant effects, >3.3 LOD, for all seed production traits examined based on overall trait means in the TTC1 family (Fig. 1, Table 3).

Interval mapping scans on the number of florets per inflorescence initially detected two main effects located on LG2a and LG4Xm (Fig. 1). An additional QTL located on LG2b was detected using the LG2a and LG4Xm QTL markers as cofactors in an rMQM scan (Fig. 1, Table 3). The LG2a, LG2b, and LG4Xm QTL markers explain 30.8% of the total variation in the number of florets per inflorescence, combined over years.

Interval mapping scans on percent seed set detected one main effect on LG7a (Fig. 1), and no additional seed set QTLs were detected using the LG7a QTL markers as cofactors in an rMQM scan (Fig. 1, Table 3).

Interval mapping scans on seed mass detected one main effect located on LG2a (Fig. 1), and no additional seed mass QTLs were detected using the LG2a QTL markers as cofactors in an rMQM scan (Fig. 1, Table 3). The LG2a seed mass QTL was significant in both 2004 and 2005 field evaluations.

Interval mapping scans on percent seed shattering initially detected three main effects on LG1b, LG3b, and LG6a (Fig. 1). The LG1b effect was not significant when the LG3b and LG6a QTL markers were used as cofactors in an rMQM scan (Fig. 1). However, another seed shattering QTL was detected on LG4Xm using LG3b and LG6a QTL markers as cofactors in the rMQM scan (Fig. 1, Table 3). The LG3b and major-effect LG6a seed shattering

Table 1. Average trait values (± SE) for the *Leymus cinereus* Acc:636 accession, *L. triticoides* Acc:641 accession, *L. triticoides* Acc:641 × *L. cinereus* Acc:636 TC1 hybrid genotype, the *L. triticoides* Acc:641 T-tester genotype, and the *L. triticoides* T-tester × (*L. triticoides* × *L. cinereus* TC1) TTC1 quantitative trait loci mapping family.

Trait	Source of TC1 hybrid parents		Parents of TTC1 family		164-sib mapping family		
	Acc:636	Acc:641	TC1	*t* tester	TTC1 overall	TTC1 min	TTC1 max
Florets per inflorescence 2006	2687 ± 210	247 ± 20	863 ± 44	214 ± 15	432 ± 14	126 ± 75	889 ± 75
Percent seed set 2005	44.3 ± 4.4	43.6 ± ND†	20.3 ± 0.9	ND	6.4 ± 0.4	0.2 ± 2.2	29.6 ± 2.2
Seed mass (mg) 2004	3.76 ± 0.07	3.48 ± 0.08	4.39 ± 0.05	3.06 ± 0.07	3.30 ± 0.03	2.15 ± 0.19	4.41 ± 0.13
Seed mass (mg) 2005	3.30 ± 0.03	2.27 ± 0.03	3.72 ± 0.02	2.84 ± 0.03	2.95 ± 0.03	2.05 ± 0.06	4.07 ± 0.06
Percent seed shatter 2005	100 ± ND	0.0 ± ND	100 ± ND	0 ± ND	43.1 ± 3.0	0.0 ± 11.7	100 ± 11.7
Percent seed shatter 2006	80.0 ± 0.11	0.0 ± 0.0	100 ± 0.0	0.0 ± .0	25.0 ± 2.9	0 ± 21.9	100 ± 21.9
Seed dormancy‡ 2004	5.4 ± 0.4	8.9 ± 0.4	6.7 ± 0.4	7.1 ± 0.4	7.9 ± 0.2	4.8 ± 0.9	12.6 ± 0.9
Seed dormancy‡ 2005	5.2 ± 0.2	6.5 ± 0.3	7.1 ± 0.1	6.1 ± 0.2	6.5 ± 0.1	3.5 ± 0.6	12.4 ± 0.6

†ND, not determined.

‡Days required to reach 50% germination (DRG_{50}).

Table 2. Phenotypic correlations between field replications of the *Leymus triticoides* × (*L. triticoides* × *L. cinereus*) TTC1 family (bold diagonal), phenotypic correlations between measurements of the same trait over different years (above diagonal), and pairwise phenotypic correlations among different traits (below diagonal).

Trait	Florets per inflorescence 2006	Seed set 2005	Seed mass 2004	Seed mass 2005	Seed shatter 2005	Seed shatter 2006	Seed dormancy 2004	Seed dormancy 2005
Florets per inflorescence 2006	0.62***	–	–	–	–	–	–	–
Percent seed set 2005	NS†	0.75***	–	–	–	–	–	–
Seed mass 2004	0.25**	0.20*	0.75***	0.82***	–	–	–	–
Seed mass 2005	0.19*	NS	–	0.89***	–	–	–	–
Percent seed shatter 2005	NS	NS	0.27***	NS	0.84***	0.65***	–	–
Percent seed shatter 2006	0.25**	NS	0.30***	NS	–	0.49***	–	–
Seed dormancy‡ 2004	NS	NS	NS	NS	0.26**	NS	0.63***	0.69***
Seed dormancy‡ 2005	NS	NS	NS	NS	0.55***	0.25**	–	0.82***

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

*** Significant at 0.001 probability level.

†NS, nonsignificant.

‡Days required to reach 50% germination (DRG_{50}).

QTLs were significant in all 2005, 2006, and combined analyses, with or without other QTL marker cofactors. The LG4Xm QTL was not significant in the 2006 evaluation. The LG3b, LG4Xm, and major-effect LG6a QTL markers explained a total of 62.5% of the total variation in percent seed shattering, combined over years.

Interval mapping scans on seed dormancy detected three main effects located on LG2a, LG6a, and LG7a (Fig. 1). However, the LG7a QTL was not significant when the LG2a and LG6a QTL markers were used as cofactors in the rMQM scan (Fig. 1, Table 3). The LG2a and LG6a seed dormancy QTLs were significant in both 2004 and 2005 and explained 34.1% of the total seed dormancy variation, combined over years.

The LG6a seed shattering and seed dormancy QTL peaks were located in precisely the same positions (Fig. 1, Table 3). This *L. cinereus* allele of this seemingly pleiotropic LG6a QTL increased percent seed shattering and decreased seed dormancy (DRG_{50}), which is consistent with trait correlations (Table 2). Rice chromosomes 1, 7, 8, and 11 are known to harbor QTL affecting both shattering and dormancy (Cai and Morishima, 2000; Ji et al. 2006), but these not syntenic with the locus reported here. Although LG2a displayed QTLs controlling the number of florets per inflorescence, seed mass, and seed dormancy, the positions of these three QTL peaks were not the same (Fig. 1). Likewise, LG4Xm displayed QTLs controlling the number of florets per inflorescence and percent seed shatter but the positions of these two QTL peaks were not the same (Fig. 1).

Leymus LG6a Seed Shatter QTL Validation Test

Interval mapping scans detected significant percent seed shatter QTL effects (LOD = 14.9) in the TTTC1.168 advance backcross population, which coincide with the TTC1 major-effect LG6a QTL on the *Leymus* LG6a integrated EST consensus map (Fig. 2). In particular, the best TTC1 and TTTC1.168 QTL markers are located in a 15.1-cM interval between E41M47.385 (67.7 cM) and E41M61.399 (82.8 cM) (Fig. 2). This LG6a QTL explained 35.2% of the total seed variation in the TTTC1.168 advance backcross population. On a scale of 0 (no shattering) to 4 (complete shattering), TTTC1.168 carriers of the *L. cinereus* LG6a seed shattering QTL allele showed an average of 1.9 units more shattering than other progeny that were homozygous for the *L. triticoides* seed retention QTL allele.

Cross-Species Alignments of Major-Effect *Leymus* LG6a Seed Shatter QTL

Five *Leymus* EST markers (Ltc92, Ltc96, *PAL*-1, *PAL*-2, and Ltc103) in the 15.1-cM LG6a seed shatter QTL align to a 2.47-megabase (Mb) region between sequences homologous to Ltc92 (23.095059 Mb) and Ltc103 (25.561310 Mb) on the rice chromosome 2 physical map (Fig. 2).

The major-effect *Zizania* group 2 seed shatter QTL peak spanned a 99-cM interval, including five restriction fragment length polymorphism (RFLP) markers CDO686, RG139b, UMC305, CDO1387, and CDO580, with peak effects located on the UMC305 locus (Kennard et al., 2002). *Zizania* linkage group 2 was first identified using 10 RFLP markers, including five markers that were previously mapped to *Oryza* linkage group 2 (Kennard et al., 2000). The *Zizania* and *Oryza* genera belong to the same tribe (Oryzeae) and show relatively close phylogenetic relationships (Ge et al., 2002). Our results show that seven of the 10 RFLP markers mapped to *Zizania* group 2 could be aligned to *Oryza* group 2, including the RZ717b, UMC305, and RG139 markers associated with the major-effect *Zizania* seed shatter QTL peak (Fig. 2). Although RZ717b was not used for QTL mapping (Kennard et al.,

2002), it maps between CDO1387 and the QTL peak on UMC305 (Kennard et al., 2000). Both CDO1387 and UMC305 showed major shattering effects in *Zizania*. Thus, the major-effect RZ717b, UMC305, and RG139 *Zizania* seed shatter QTL markers align to a 3.56-Mb region between the RZ717 and RG139. The major-effect *Leymus* and *Zizania* seed shatter QTLs show about 52% overlap based on alignments to the *Oryza* genome. Seed shattering in *Zizania* reportedly occurs beneath the spikelets, but there is only one floret per spikelet and glumes are absent in *Zizania* (Barkworth et al., 2007). Thus, it is difficult to determine whether or not the *Zizania* and *Leymus* seed abscission layers are located in homologous structures, as the resulting diaspores are structurally homologous.

Table 3. Summary of quantitative trait loci (QTLs) marker effects detected using restricted multiple QTL model scans of the high-density *Leymus* TTC1 genetic map described by Wu et al. (2003) as shown in Fig. 1.

Trait	Linkage group	QTL marker (cM position) interval	LOD†	Percent variation explained	Effect‡
Florets per inflorescence	LG2a	P33M50.249 (56.4)	7.3	15.9	138
Florets per inflorescence	LG2b	E37M60.186 (137.1)–E36M49.092 (145.7)	4.2	9.4	103
Florets per inflorescence	LG4Xm	E41M49.343 (47.3)–E35M61.332 (52.2)	4.5	10.2	113
Percent seed set	LG7a	E36M49.061 (14.4)–E41M62.212 (25.9)	3.4	10.1	–3.5
Seed mass (mg)	LG2a	E41M47.356 (131)–P42M61.185 (134)	3.9	11.8	0.272
Percent seed shatter	LG3b	E37M47.326 (117.8)	6.1	7.1	18.4
Percent seed shatter	LG4Xm	E36M48.118 (77.0)–E41M62.369 (80.4)	4.5	5.5	16.4
Percent seed shatter	LG6a	E38M49.336 (71.2)–P33M48.386 (73.2)	26.9	43.1	45.8
Seed dormancy§	LG2a	P35M62.365 (37.1)–E36M59.221 (38.8)	3.9	10.2	–1.05
Seed dormancy§	LG6a	E38M49.336 (71.2)–P33M48.386 (73.2)	10.6	24.1	1.64

†LOD, log of odds likelihood ratio.

‡Average trait value of genotypes that were heterozygous for *L. cinereus* and *L. triticoides* alleles minus the average trait value of genotypes that were homozygous for *L. triticoides* allele.

§Days required to reach 50% germination (DRG_{50}).

One of three QTLs explaining 24% of the seed shattering variation in *Oryza sativa* was associated with six RFLP markers in linkage group 2 (Paterson et al., 1995a). Sequences from five of these six RFLP probes (rz58, rz567, CDO686, CDO204, and rz446) were aligned relative to other *Leymus* and *Zizania* marker sequences on the *Oryza* chromosome 2 physical map (Fig. 2). The *Oryza* seed shatter QTL peak (Paterson et al., 1995a) was located between CDO686 and CDO204. Thus, there is some overlap of seed shatter QTL effects between the *Leymus*, *Oryza*, and *Zizania* seed shatter QTL alignments on rice chromosome 2 (Fig. 2). However, it is again difficult to determine whether or not the *Oryza*, *Leymus*, and *Zizania* seed abscission layers are located in homologous structures. The genus *Oryza* has three florets per spikelet, but only the terminal floret is functional (Barkworth et al., 2007). Although the *Oryza* seed abscission layer occurs above the glume, the resulting diaspore includes lemmas from each of the two reduced (sterile) florets subtending the palea and lemma of the terminal floret and caryopsis (Ji et al., 2006; Barkworth et al., 2007).

Doebley and Stec (1993) compared QTLs controlling seed disarticulation in two families derived from crosses of domesticated maize and wild teosinte (*Zea mays* ssp. *mays* × *Z. mays* ssp. *parviglumis*). A total of six seed disarticulation QTLs were detected in the Chapalote maize *Zea mays* × *Z. mays* ssp. *mexicana* teosinte (CM) family. A total of four seed disarticulation QTLs were detected in the Reventador maize *Zea mays* × *Z. mays* ssp. *parviglumis* teosinte (RP) family. Only one of these nine QTLs, located on chromosome 4, was considered the same between RP and CM families (Doebley and Stec, 1993; Doebley, 2004). Paterson et al. (1995a) aligned the *Zea* chromosome 4 seed disarticulation QTLs, detected in both RP and CM families, to the *Oryza* chromosome 2 seed shattering QTL shown in Fig. 2. Although most available maize chromosome 4 sequences align to rice chromosome 2, there are many pair-wise exceptions (Ahn and Tanksley, 1993; Salse et al., 2004). Moreover, the CM and RP *Zea* linkage maps comprise a relatively small number of RFLP markers, most of which are maize genomic DNA clones. Thus, it is difficult to align these maps directly to the rice physical map shown in Fig. 2. Nevertheless, the CM *Zea* seed disarticulation QTL was located near the UMC42A locus (Doebley and Stec, 1993), which was closely linked (0 or 3.3 cM) to the *teosinte glume architecture 1* (*tga1*) locus in two mapping families (Dorweiler et al., 1993). We aligned the *tga1* gene (Wang et al., 2005) near the centromere of rice chromosome 2 (Fig. 1). Based on these results and other alignments (results not shown) the *Zea* chromosome 4 seed disarticulation QTL shows correspondence with the *Leymus* and *Zizania* QTLs.

Cross-Species Alignments of *Leymus* LG3 Seed Shatter QTL

A discrete spikelet disarticulation gene, *Br2*, was mapped to a distal region on the long arm of homeologous group 3 in a cross of shattering AL8/78 and nonshattering TA1604 variants of *Aegilops tauschii* Coss. (Triticeae) (Li and Gill, 2006). The *Ae. tauschii Br2* gene was aligned to a 2.8-Mb interval between sequences homologous to the wheat *XBE499968* and *Xpsr170* markers, on the distal side of the *qSH1* gene on rice seed shattering gene (Li and Gill, 2006). Although the wheat *Br2* gene may align to the *Leymus* 3b

shattering QTL (Fig. 3), the rice *qSH1* gene (Konishi et al., 2006) does not align with this *Leymus* QTL region (Fig. 3). Although most seed shattering in *Leymus* can be attributed to abscissions that occur above the glume and below the floret, we did observe some spikelet disarticulation. Thus, it is possible that the relatively minor *Leymus* LG3 shattering QTL involves spikelet disarticulation homologous to wheat.

CONCLUSIONS

Genomewide DNA marker analyses detected independent QTL effects for all five traits and one ostensibly pleiotropic QTL with major effects on seed shattering and seed dormancy. The most important outcome of this study is discovery of the pleiotropic QTL on *Leymus* LG6a with major effects on both seed shattering and seed dormancy. Unexpectedly, the creeping wildrye allele for this major-effect LG6a QTL actually decreased seed dormancy (DRG_{50}). Creeping wildrye is known for having extensive seed dormancy and did in fact show relatively slow germination rates in our study. We deduce that the LG6a creeping wildrye seed retention gene decreased seed dormancy of the TTC1 seeds by enabling more complete natural seed ripening under typically hot, dry conditions of its native environments. However, we do not believe that the LG6a seed dormancy QTL was simply caused by differences in harvest time because we also detected the same gene effects in 2004, when seed was harvested by clipping, as plants senesced, regardless of whether or not it was shattering. Moreover, *Leymus* LG6a was not associated with maturity variation as measured by date of anthesis in the TTC1 family (Larson et al., 2006). Thus, we hypothesize that the dominant LG6a basin wildrye seed shattering gene enables formation of a seed abscission layer, which hinders natural seed development and seed ripening in the creeping wildrye backcross population. However, we did not detect any other pleiotropic effects of this major-effect seed shattering gene on the number of florets per inflorescence, percent seed set, or seed mass. Thus, in addition to eliminating the possibility of seed shattering losses, the *L. triticoides* seed retention allele will facilitate more complete seed development and ripening of cultivated basin wildrye seed crops. Molecular markers associated with this seed retention gene allele have been implemented in marker-assisted selection experiments involving an admixed *L. cinereus* × *L. triticoides* breeding population described by Hu et al. (2005). These markers may also have application in closely related species, including Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski] and western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve], which are known to have problematic seed shattering characteristics (Berdahl and Frank, 1998).

The major-effect *Leymus* LG6a seed shatter QTL aligns to another major-effect seed shatter QTL in American wildrice and other possible seed shatter QTLs in *Oryza*, *Zea*, and *Triticum*. Thus, identification of this gene would be an important breakthrough in grass breeding and genetic research. Relatively new *Leymus* genetic maps, EST resources, and 5.3X BAC genomic DNA library (Wu et al., 2003; Larson et al., 2007; Bushman et al., 2008; Kaur et al., 2008) provide a potentially useful platform for fine-genetic mapping and positional cloning of the dominant basin wildrye seed shattering gene in creeping wildrye backcross populations.

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